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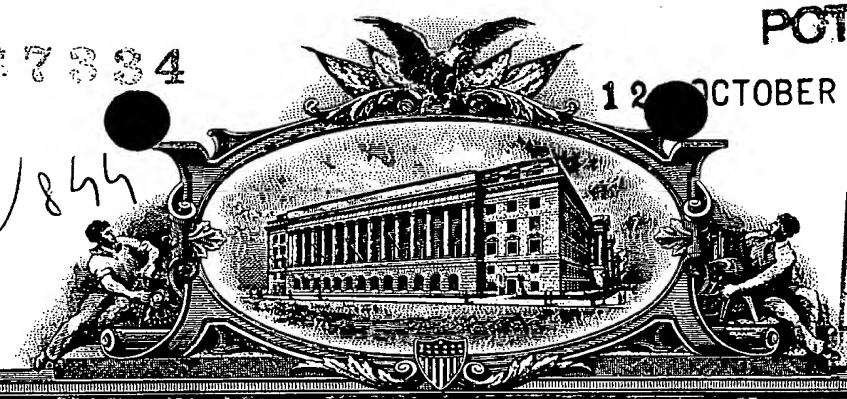

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United States Patent and Trademark Office

September 16, 1999

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APPLICATION NUMBER: ***09/154,627***
FILING DATE: ***September 17, 1998***

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

File No. 12667-16"US" FC/ld

JC511 U.S. PTO
09/154627
09/17/98

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09/17/98

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Sylvain CHEMTOB et al.

For: G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

Your petitioner prays that letters patent may be granted for the invention set forth in the enclosed specification including a disclosure, claims and declaration.

Enclosed are :

☒ 4 sheet(s) of drawings.

☒ An additional copy of this sheet and an Assignment of the Invention to HÔPITAL SAINTE-JUSTINE

☐ A certified copy of _____ on the basis of which the benefit of priority under 35 U.S.C. 119 is claimed.

☒ Declaration of small entity status.

	No. filed	Number Extra	Rate	Basic Fee $395.00
Total Claims	7			-
Multiple Dependency Fee	-	-	-	-
Independent Claims	1			-

Base Filing Fee	$395.00
Assignment Fee	40.00
Total	$435.00

A cheque No. 005870 including the amount of $435.00 to cover the Government Filing and Extra Claims Fee is enclosed.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Account No. 19-5113.

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Date: September 16, 1998

G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to development of antagonists of a G protein-coupled receptor which bind to the G protein-coupled receptor from the extracellular side.

(b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomelular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* **9**:1051-10585). Prostanoids mediate their actions through binding to distinct receptors which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G-proteins comprising of α, β and γ subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* **63**:101-132).

Of the five pharmacologically distinct prostanoid receptors for PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and TxA_2 and their many isoforms, the receptor for $PGF_{2\alpha}$, also called FP receptor, shows limited tissue distribution, predominantly expressed in corpora leutea, uterine

myometrium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is marked by tremendous rise in $PGF_{2\alpha}$ levels and increased uterine contractility. The wide spread use of $PGF_{2\alpha}$ analogues to induce labor in veterinary industry points to the primary role of $PGF_{2\alpha}$ and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto et al., 1997, *Science* **277**:81-83). In face of escalating costs incurred as a result of premature births and associated complications to the neonate, such as intraventricular hemorrhage, bronchopulmonary displasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature labor is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly PGE_2 and $PGF_{2\alpha}$. However, inhibition of PGE_2 is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension.

At another level, $PGF_{2\alpha}$ has been attributed a major role in dysmenorrhea, a condition which afflicts 5%-7% of premenopausal women. A pre-menstrual increase in $PGF_{2\alpha}$ levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended therapy hampered the advances in preventing premature labor and associated sequelae, and the design of such antagonists is the subject of this application.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares only 47% sequence identity with EP_1 receptor, and to a

lesser extent with other prostanoid receptors (Abramovitz et al., 1994, *J. Biol. Chem.* **269**:2632-2636). Binding of $PGF_{2\alpha}$ to FP receptor is followed by the activation of $G_{\alpha q\beta\gamma}$ complex, increased GTP binding by the $G_{\alpha q}$ subunit, stimulation of phospholipase Cβ activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* **46**:205-229). The FP receptor is the only efficacious target for development of therapeutic drugs since a few G_{α}-proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors. Their efficacy will be compromised in face of tremendous increase in $PGF_{2\alpha}$ concentrations in myometrium at the onset of labor and in menstruation. The high basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

It would be highly desirable to be provided with antagonists of FP receptors which do not crossreact with other prostanoid receptors and which are effective even in the presence of excess ligand.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide antagonists of FP receptors which do not crossreact with other prostanoid receptors and which are effective even in the presence of excess ligand.

Another aim of the present invention is to provide inhibitors of FP receptors by a novel strategy to target the extracellular domains of the receptor protein.

In accordance with the present invention, there is provided a G protein-coupled receptor antagonist which specifically binds to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.

In accordance with a preferred embodiment of the present invention, the antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.

In accordance with a preferred embodiment of the present invention, the G protein-coupled receptor is the FP receptor of prostaglandin $F_{2\alpha}$.

In accordance with a preferred embodiment of the present invention, the antagonists of the present invention comprise amino acid sequences derived from the prostaglandin $F_{2\alpha}$ receptor. Preferably, the antagonists include, without limitation, amino acid sequences of the FP receptor ILGHRDYK (PCP-8; SEQ ID NO:1) or WEDRFYLL (PCP-10; SEQ ID NO:2), protein fusions or peptidomimetic thereof.

In accordance with another embodiment of the present invention, the antagonists of the present invention comprise amino acid sequence derived from the second extracellular loop of prostaglandin or thromboxane receptors.

In accordance with another embodiment of the present invention, there is provided a method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional

derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor, thereby hampering uterine contractions.

In accordance with another embodiment of the present invention, there is provided a method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor to hamper transduction of a signal thereby reducing the pain associated with contractions.

For the purpose of the present invention the following terms are defined below.

The expression "a G protein-coupled receptor antagonist" is intended to mean any natural or synthetic compound, peptide protein, antibody, peptidomimetic or small chemical molecules, without limitation, insofar as it can specifically bind to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal. More preferably, the antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.

The expression "functional derivatives" of G protein-coupled receptor antagonist is intended to mean mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled receptor antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the inhibitory effects of PCP-8 and PCP-10 on FP receptor function upon stimulation with $PGF_{2\alpha}$ in accordance with the embodiment of the present invention;

Fig. 2A illustrates the effects of PCP-8 and PCP-10 on the diameter of the microvessels of pig retina upon stimulation with either $PGF_{2\alpha}$ or thromboxane A_2 mimetic, U46619;

Fig. 2B illustrates the dose response of $PGF_{2\alpha}$ on the diameter of pig microvessels treated previously with PCP-8 or PCP-10;

Fig. 2C illustrates the effects of thromboxane A_2 mimetic, U46619, on the diameter of pig microvessels treated previously with PCP-8 and PCP-10;

Fig. 3A illustrates the effects of PCP-10 upon spontaneous contractions of uterine smooth muscle;

Fig. 3B illustrates the dose response of prostaglandin $F_{2\alpha}$ in the presence/absence of PCP-8 and PCP-10 upon uterine smooth muscle contraction; and

Fig. 4 illustrates the reversal of basal tone of bovine myometrium even in the presence of FP receptor ligand, $PGF_{2\alpha}$.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a new class of G protein-coupled receptor antagonists which bind to the extracellular molecular surface, thus hamper signal transduction.

Also provided is a novel strategy to target the extracellular loops of the receptor which contribute to the structural or functional integrity of the receptor. Antagonists thus bind to cognate elements in the extracellular surface of the receptor and prevent the

receptor function by interfering with its signal initiation or transduction.

There is provided proof of selectivity of the antagonists to FP receptor by showing an absence of their effects on a related prostaglandin receptor for thromboxane A_2, known as TP receptor which is also involved in smooth muscle contraction.

Preparation of inhibitors

Chemical synthesis of PCP-8 and PCP-10:

We have synthesized using F-moc chemistry and solid phase Merrifield method two peptides, PCP-8 and PCP-10 which are 8 amino acids in length. These peptides were purified by HPLC and their purity tested by mass spectroscopy.

In accordance with the present invention, a novel strategy of using peptides derived from the extracellular domains of prostaglandin $F_{2\alpha}$ receptor, FP, to inhibit the signal transduction and the functional consequences of FP receptor. This method could be generalized to all G protein-coupled receptors. Peptides derived from the second extracellular domain of FP receptor were found to be effective inhibitors of FP receptor.

The present invention could be readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Effects of peptides, PCP-8 and PCP-10, on ligand-induced phosphoinositide hydrolysis in mammalian cells overexpressing the FP receptor

Both PCP-8 and -10 were tested in HEK293 cells expressing the human FP receptor. For this purpose, HEK 293 cells stably expressing human FP receptor were plated in 12-well plates in DMEM medium containing 10%

fetal bovine serum, penicillin (10 U/ml) and streptomycin (10 μg/ml) and cultured in a humidified atmosphere containing 5% CO_2 at 37°C. After the wells were 80% confluent, the cells were labeled with 2 μCi/ml of [^{3}H]-*myo* inositol overnight. Next day, the cells were washed once with PBS, and incubated in 0.5 ml of Kreb's buffer containing 10 mM LiCl and indicated concentrations of PCP peptides for 30 min. $PGF_{2\alpha}$ at 1μM was added to the cells and the incubation was carried out for an additional 30 min. The cells were solubilized with 0.1 N NaOH for 10 min and neutralized with 0.1 N formic acid. The lysates were collected and 1 ml each of methanol and chloroform were sequentially added and vortexed briefly. After centrifugation to separate the phases, inositol phosphates were separated by ion exchange chromatography as described below (Berridge, M.J. et al., 1983, *Biochem. J.* **212**:473-482).

Briefly, the medium was discarded and the IP3 synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation at 6000 x g for 10 min. The aqueous phase was applied to AG-1X-8™ (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III), the eluates were counted by scintillation spectrophotometry.

The results of these experiments are shown in Fig. 1. Data are expressed as fold stimulation of

inositol phosphate synthesis by 1 μM $PGF_{2\alpha}$ compared to the unstimulated controls. Both PCP-8 and -10 at 100 μM potently inhibited inositol phosphate synthesis initiated by the action of $PGF_{2\alpha}$ on FP receptor. The half maximal inhibitory concentrations for both PCP-8 and -10 were slightly less than 100 μM.

EXAMPLE II

Testing PCP peptides in porcine eyecup model of *ex vivo* vasomotricity assay

In order to see if the peptides could inhibit FP function using an *ex vivo* model, we chose porcine eyecup model, an *ex vivo* assay of vascular constriction in porcine retinas which we previously described and validated (Li et al., 1996 J. Pharmacol. Expt. Therapeut. 278: 370-377; Li et al., 1997 Am. J. Physiol. 273: R1283-90; Abran et al., 1997 Am. J. Physiol. 272: R995-1001). Since FP receptor densities in newborn vasculature are minimal due to down regulation by high levels of circulating prostaglandins in the perinatal period, the newborn pigs were treated with a prostaglandin synthase blocker, ibuprofen (30 mg/Kg of bodyweight/ 8 h for 24 h) to increase the density of the receptors as well as their vasomotor effects. By inhibiting circulating prostaglandins, we were able to show potent inhibition of FP receptor-mediated second messenger synthesis as well as FP-mediated vascular constriction in this eyecup model.

To prepare eyecups, a circular incision was made 3-4 mm posterior to ora serrata to remove the interior segment and vitreous body with minimal handling of the retina. The remaining eyecup was fixed with pins to a wax base in a 20 ml tissue bath containing 20 ml of Kreb's buffer (pH 7.35-7.45), protease inhibitors, leupetin and aprotinin (10 μg/ml each), and equilibrated with 21% oxygen and 5% carbon

dioxide at 37°C. The preparations were allowed to stabilize for 30 min. Peptides at 100 μM were added and incubation was continued for 30 min before the addition of $PGF_{2\alpha}$.

Cumulative concentration-responses of $PGF_{2\alpha}$ and TxA_2 mimetic, U46619, (10^{-10} to 10^{-5} M) curves were constructed separately. To assess the reversibility of the antagonists, the eyecups were thoroughly washed (which would wash away the peptide) with incubation medium and concentration response curves for $PGF_{2\alpha}$ were determined. The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400™) and the responses were quantified by a digital image analyzer (Sigma Scan Software, Jandel Scientific, Corte Madera, CA). Vascular diameter was recorded before and 10 min following the topical application of the agent. Each measurement was repeated three times and showed <1% variability.

The results are shown in Fig. 2. The peptide PCP-10 had no effect on the basal tone (diameter of the microvessel) of the vessel (Fig. 2A; left panels). Addition of 1 μM of $PGF_{2\alpha}$ potently constricted the vessel in the absence of the peptide (middle-top panel), whereas presence of PCP-10 at 100 μM markedly inhibited $PGF_{2\alpha}$-mediated vasoconstriction (middle-bottom panel). The peptide had no effect on the vasoconstriction effected by 1 μM TxA_2 mimetic, U46619, (right panels) acting on another prostanoid receptor coupled to constriction, namely TP receptor. Similar results were obtained for PCP-8 as well. A dose response of $PGF_{2\alpha}$ on the vascular diameter in the presence/absence of PCP-8 and PCP-10 peptides are presented in Fig. 2B. Both peptides abrogated the vasomotor responses even at concentrations exceeding 1μM of $PGF_{2\alpha}$, suggesting, as expected, that the

peptides may be acting in a non-competitive fashion. However, the peptides had no effect on vasoconstriction produced by thromboxane A_2 (Fig. 2C).

EXAMPLE III

Testing PCP peptides in porcine uterine strip of *ex vivo* basal contraction assay

In *ex vivo* experiments using porcine uterine strips, the peptides were able to prevent both basal and $PGF_{2\alpha}$-induced contraction.

Uterine tissues from non-pregnant adult pigs were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as we have described (Potvin, W. et al., 1990, *Br. J. Pharmacol.* **100**:341-347; Varma, D.R. and Chemtob, S., 1993, *J. Pharmacol. Expt. Ther.* **265**:1096-1104). Contractions were recorded by force transducers on Grass-polygraph. Strips were incubated with or without 100 µM peptides for 30 min before adding $PGF_{2\alpha}$ in step-wise increments (10^{-9} to 10^{-6} M). Data were expressed as percentage increase over the basal level of average tension (g).

A graph of spontaneous uterine contractions (known to be dependent upon prostanoids, mainly $PGF_{2\alpha}$) in the absence and the presence of 100 µM PCP-8 are shown in Fig. 3A. Addition of peptide to the strips reduced the force of basal contraction. A dose response of $PGF_{2\alpha}$ on uterine contractility in the presence or absence of PCP-8 and PCP-10 peptides is shown in Fig. 3B. More than 60% (PCP-8) and 80% (PCP-10) reduction in uterine contraction was observed in all concentrations of $PGF_{2\alpha}$ tested. Thus, both these peptides reduced spontaneous as well as $PGF_{2\alpha}$-induced contractions in the uterine strips.

EXAMPLE IV

Testing PCP peptides in bovine uterine strip of *ex vivo* basal contraction assay

Uterine tissues from non-pregnant adult bovine animals were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as described above. Contractions were recorded on Grass-polygraph by force transducers. Strips were incubated with or without 100 μM peptides before adding $PGF_{2\alpha}$ in step-wise increments (10^{-8} to 10^{-6} M). Data were expressed as change in basal level of average tension (g). The results are shown in Fig. 4. Application of PCP-10 peptide at 100 μM reversed the basal tone (contractile state) of the uterine muscle. Addition of $PGF_{2\alpha}$ up to 10μM did not affect the relaxation produced by PCP-10 suggesting that the effects of PCP peptides are independent of the ligand, which was also shown in the previous results.

While the invention has been described in connection with specific embodiment thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come within the known customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

SEQUENCE LISTING

<110> Hôpital Sainte-Justine
CHEMTOB, Sylvain
PERI, Krishna G.

<120> G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

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WHAT IS CLAIMED IS:

1. A G protein-coupled receptor antagonist which specifically binds to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.

2. The antagonist of claim 1, wherein said antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.

3. The antagonist of claim 1, wherein the receptor is prostaglandin $F_{2\alpha}$ receptor (FP receptor).

4. The antagonist of claim 2, which comprises amino acid sequences of the FP receptor ILGHRDYK (PCP-8; SEQ ID NO:1) or WEDRFYLL (PCP-10; SEQ ID NO:2), protein fusions or peptidomimetic thereof.

5. The antagonist of claim 1, which comprises an amino acid sequence derived from the second extracellular loop of prostaglandin or thromboxane receptors.

6. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 5 or functional derivatives thereof.

7. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically

effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 5 or functional derivatives thereof.

ABSTRACT OF THE INVENTION

The present invention relates to a new class of G protein-coupled receptor antagonist which specifically binds to the receptor protein structural elements, thus hampering ligand-initiated signal transmission and subsequent physiological effects such as smooth muscle contraction. Described herein are peptide sequences derived from the G protein-coupled receptor protein, produced by chemical methods as selective inhibitors of signal transduction associated with stimulation of the receptor by its ligand. Such peptides or molecules derived from their primary, secondary or tertiary structures may be used as effective tocolytics for the prevention of premature labor or be used for the treatment of dysmenorrhea.

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled G PROTEIN-COUPLED RECEPTOR ANTAGONISTS, the specification of which

[X] is attached hereto.

[ ] was filed on _____ as Application No. _____
and (if applicable) was amended on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having filing date before that of the application on which priority is claimed;

Prior Foreign Application(s)

Number	Country	Day/Month/Year Filed	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Day/Month/Year Filed	Status (Patented, Pending, Abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.
ROBERT MITCHELL, Registration No. 25,007, GUY HOULE, Registration No. 24, 971, PAUL MARCOUX, Registration No. 24,990, KEVIN P. MURPHY, Registration No. 26,674; ROBERT CARRIER, Registration No. 30,726; MICHEL J. SOFIA; Registration No. 37,017; FRANCE CÔTÉ, Registration No. 37,037; and JAMES ANGLEHART, Reg. No. 38,796, and address all correspondence to:

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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full name of second inventor	**Inventor's signature**	**Date**
Krishna G. PERI		16/09/98
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Applicant or Patentee: Sylvain Chemtob et al.
Serial or Patent No.: _____ Atty. Dkt. No.: 12667-16"US" FC/ld
Filed or Issued: _____
For: G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS [(37 CFR 1.9(f) AND 1.27 (d)] - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION HÔPITAL SAINTE-JUSTINE
ADDRESS OF ORGANIZATION 3175 chemin de la Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5
TYPE OF ORGANIZATION

(X) UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
( ) TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(A) AND 501(C)(3)]
( ) NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
( ) WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(A) AND 501 (C)(3)] IF LOCATED IN THE UNITED STATES OF AMERICA
( ) WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9 (e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention entitled G PROTEIN-COUPLED RECEPTOR ANTAGONISTS by inventor(s) Sylvain CHEMTOB and Krishna G. PERI described in

(X) the specification filed herewith
( ) application serial no. _____, filed _____
( ) patent no. _____ issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). *NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. [37 CFR 1.27]

NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION
NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Richard Maheu
TITLE IN ORGANIZATION Assistant Director
ADDRESS OF PERSON SIGNING 3175 Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5

SIGNATURE _____ DATE 16 sept '98

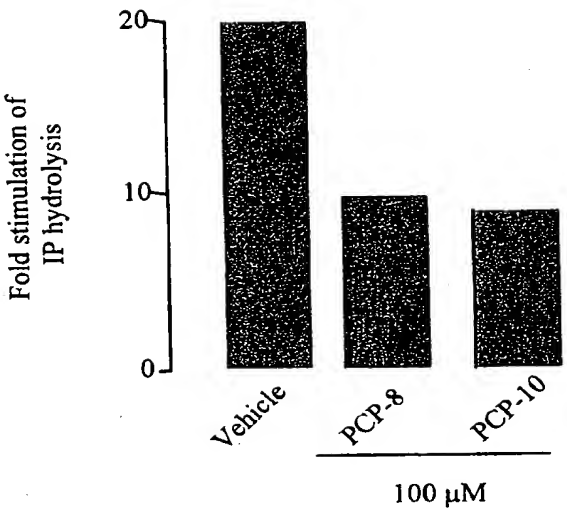
Fold stimulation of
IP hydrolysis
20
10
0
Vehicle
PCP-8
PCP-10
100 μM

Fig. 1

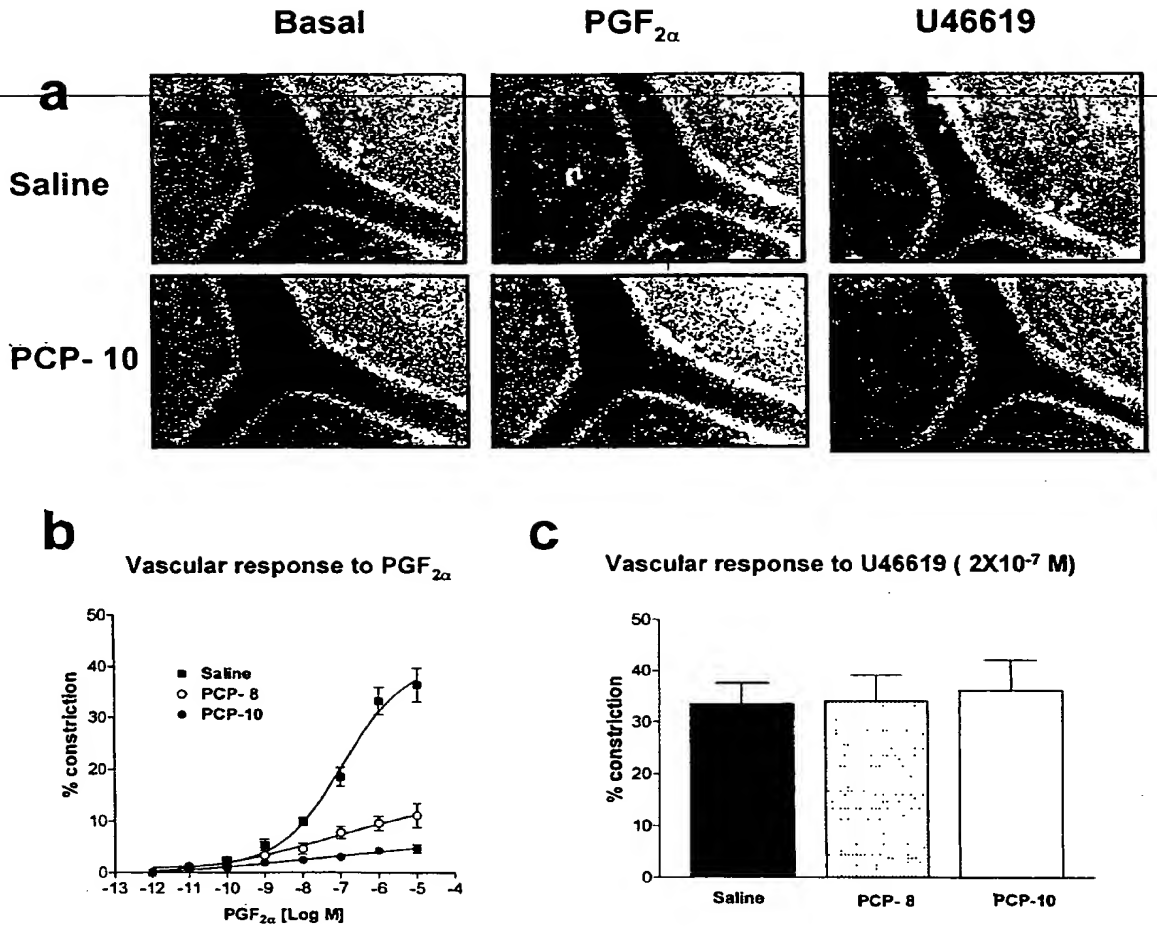
Basal
PGF2α
U46619
a
Saline
PCP- 10
b
Vascular response to PGF2α
% constriction
Saline
PCP- 8
PCP-10
PGF2α [Log M]
c
Vascular response to U46619 (2X10-7 M)
% constriction
Saline
PCP- 8
PCP-10

Fig. 2

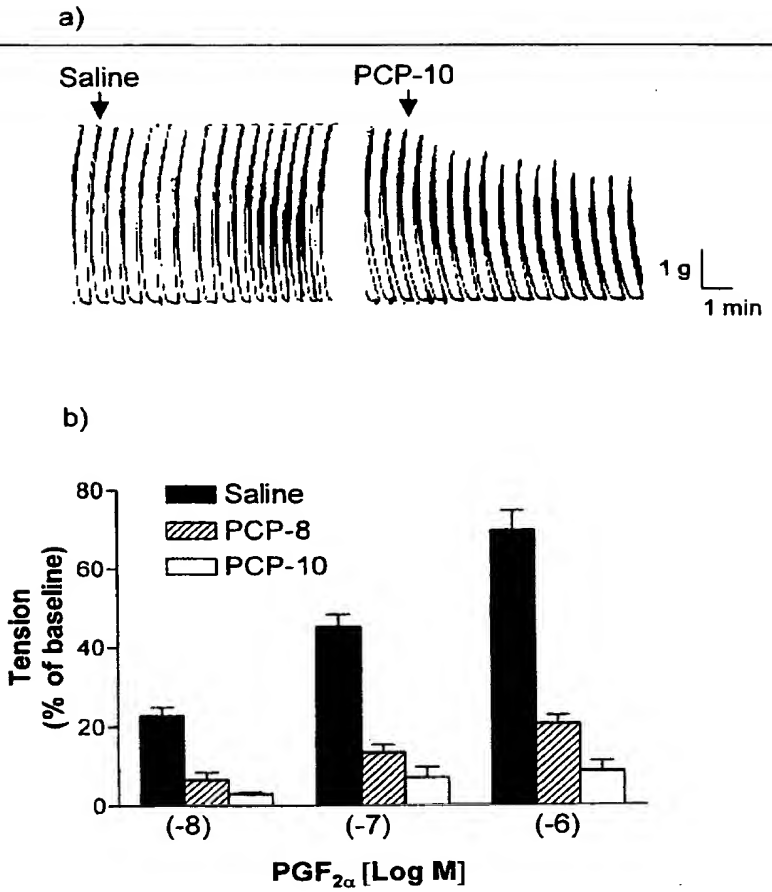
a)
Saline
PCP-10
1 g
1 min
b)
Saline
PCP-8
PCP-10
Tension
(% of baseline)
80
60
40
20
0
(-8)
(-7)
(-6)
PGF2α [Log M]

Fig. 3

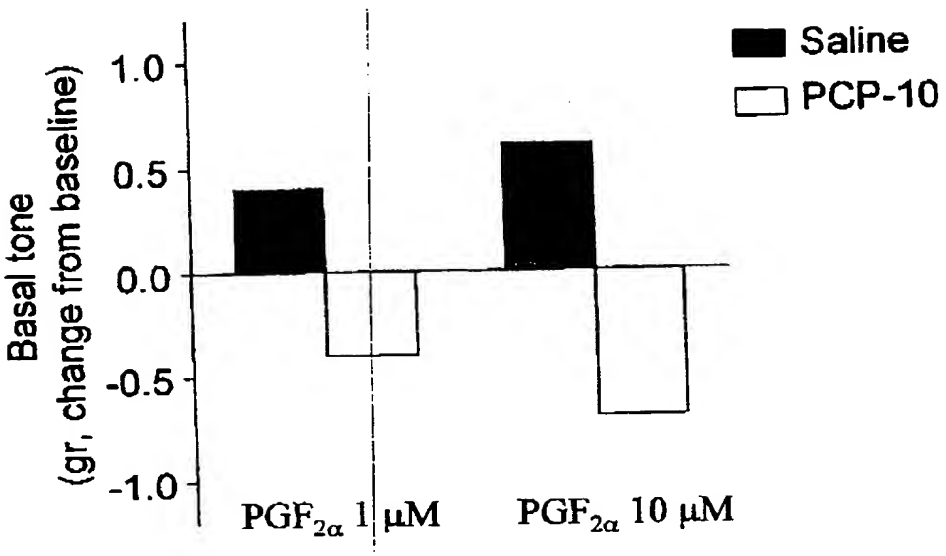
Saline
PCP-10
Basal tone
(gr, change from baseline)
1.0
0.5
0.0
-0.5
-1.0
PGF2α 1 μM
PGF2α 10 μM

Fig. 4